in following the metabolism in serum (diluted 1-10) of some of the esters mentioned above (7). The hydrolysis of the ester could be determined without interference from its quaternary metabolite since only those esters were selected for this study which did not form metabolites belonging to Group I.

Summary. A general method is presented for the determination in buffer solutions, nerve homogenates, dilute serum, plasma, and urine of quaternary ammonium compounds in which at least one chain is 4 carbons or longer or $C_6H_5CH_2$—, or modifications thereof. The lower limit of sensitivity, using this procedure, is about 2-3 μg per sample. Adaptations of this method allow for the estimation of these compounds in undiluted serum or plasma. Acetylcholine, hexamethonium, tetraethylammonium, neostigmine and *d*-tubocurarine can be determined in certain media, in concentrations as low as 1-2.5 μg per sample, by means of modifications of the general procedure.

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Anaphylactic Shock in the Pertussis Vaccinated Mouse. II. Diphtheria and Tetanus Toxoids as Antigens.* (19792)

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The observation(1,2) that mice immunized by a mixture of *H. pertussis* organisms and horse serum become markedly anaphylactogenic to a challenging dose of the serum led us to study other antigens. The effect of pertussis vaccination on increasing the sensitizing capacity of diphtheria and tetanus toxoids was particularly interesting because of reported accidents, such as the clinical report(3) of fatal anaphylaxis following reinjection of a pertussis-diphtheria toxoid vaccine.

Methods. The technic utilized was that previously described(1,2). Female, white mice were inoculated intraperitoneally with 0.5 ml of a mixture containing about 8,750 million phase I organisms of *H. pertussis* vaccine, Sharp and Dohme, and 0.1 ml of either diphtheria or tetanus toxoid. The toxoids incorporated in this mixture were as follows: diphtheria toxoid, fluid; diphtheria toxoid, alum precipitated; tetanus toxoid, fluid; tetanus toxoid, alum precipitated.† Control groups of mice receiving only the diphtheria toxoid were injected at the same time as the experimental group. Fifteen days after immunization the mice were challenged by the intravenous injection of 0.1 ml of the respective fluid toxoid. The mortality rate was determined as the number of animals dead after 24 hours/number of animals injected.

Results and discussion. The results are tabulated in Table I. It can be seen that *H. pertussis* vaccine enhances the anaphylactogenicity of fluid diphtheria toxoid but apparently has no effect on tetanus toxoid. It was not deemed necessary to challenge the tetanus toxoid control groups since it had already been observed that the toxoid com-

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† The toxoids used in this study were Diphtheria Toxoid, Illinois Department of Public Health; Diphtheria Toxoid, Alum Precipitated, Lilly; Tetanus Toxoid Fluid, Lilly; and Tetanus Toxoid, Alum Precipitated (Refined), Wyeth.

TABLE I. Enhancing Effect of *H. pertussis* Vaccine on Anaphylactogenicity of Various Toxoids; Mortality Rate = No. of Animals Dead after 24 Hr/No. of Animals Challenged.

	Toxoid alone	%	Toxoid combined with pertussis	%
Mortality in mice challenged with toxoid intrav. 15 days after intraper. inj. of				
Diphtheria, fluid	2/40	5	25/33	76
" , alum precipitated	12/38	31	14/26	54
Tetanus, fluid	—	—	4/38	11
" , alum precipitated	—	—	1/35	3
Mortality in mice challenged with toxoid intrav. 10 days after intraper. inj. of				
Diphtheria, alum precipitated	2/18	11	25/64	39
Tetanus, fluid	—	—	2/21	10
" , alum precipitated	—	—	0/10	0

bined with pertussis vaccine caused no increase in mortality rate. The difference in behavior between tetanus and diphtheria toxoid is perhaps a reflection of a possible difference in their antigenic capacity in the mouse.

The high mortality of the control group receiving alum precipitated diphtheria toxoid may be the combined effect of the adjuvant action of the alum acting over the 14-day period. It has been observed(5) that even after 10 days a high mortality rate results following antigenic challenge of a series of mice sensitized with a mixture of horse serum and vaccine. This shorter incubation period was utilized in another series of experiments with the tetanus toxoids and diphtheria toxoid, alum precipitated. One group of mice was injected with 0.5 ml of a solution containing 8,750 million *H. pertussis* organisms and ½ a human immunizing dose of toxoid. The control group received the toxoid alone. The animals were challenged after 10 days using 0.1 ml of the tetanus or diphtheria toxoid, fluid, given intravenously. The results also appear in Table I. The enhancement of the anaphylactogenicity of diphtheria toxoid, alum precipitated, by pertussis is more strikingly apparent. On the other hand, alteration of the size of the dose and time interval before challenge had no effect on the anaphylactogenicity of the tetanus toxoids.

It should be noted that the mortality rate in these studies is considered as the number of animals dead after 24 hours. Our observations have subsequently shown that the mortality rate can be recorded after 4 hours. Only rarely does an animal succumb after this period of time. We have made no attempt in these studies to record the appearance of signs of anaphylaxis other than the fatalities, such as described by Nelson, Fox, and Freeman(4).

Summary. 1. Mice immunized by an intraperitoneal injection of a mixture of *H. pertussis* and diphtheria toxoid become markedly anaphylactogenic to a challenging dose of toxoid. 2. Under the conditions of the experiments described pertussis vaccine has no enhancing effect on the anaphylactogenicity of tetanus toxoids.

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